More than a 'speed gene': ACTN3 R577X genotype, trainability, muscle damage, and the risk for injuries

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Abstract:	A common null polymorphism (rs1815739; R577X) in the gene that codes for α-actinin-3 (ACTN3) has been related to different aspects of exercise performance. Individuals who are homozygous for the X allele are unable to express the α-actinin-3 protein in the muscle as opposed to those with the RX or RR genotype. α-actinin-3 deficiency in the muscle does not result in any disease. However, the different ACTN3 genotypes can modify the functioning of skeletal muscle during exercise through structural, metabolic or signalling changes, as shown in both humans and in the mouse model. Specifically, the ACTN3 RR genotype might favour the ability to generate powerful and forceful muscle contractions. Leading to an overall advantage of the RR genotype for enhanced performance in some speed and power-oriented sports. In addition, RR genotype might also favour the ability to withstand exercise-induced muscle damage, while the beneficial influence of the XX genotype on aerobic exercise performance

	needs to be validated in human studies. More information is required to unveil the association of ACTN3 genotype with trainability and injury risk during acute or chronic exercise.
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Title: **More than a 'speed gene': *ACTN3* R577X genotype, trainability, muscle damage and the risk for injuries**

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ABSTRACT

A common null polymorphism (rs1815739; R577X) in the gene that codes for α-actinin-3 (*ACTN3*) has been related to different aspects of exercise performance. Individuals who are homozygous for the X allele are unable to express the α-actinin-3 protein in the muscle as opposed to those with the RX or RR genotype. α-actinin-3 deficiency in the muscle does not result in any disease. However, the different *ACTN3* genotypes can modify the functioning of skeletal muscle during exercise through structural, metabolic or signalling changes, as shown in both humans and in the mouse model. Specifically, the *ACTN3* RR genotype might favour the ability to generate powerful and forceful muscle contractions. Leading to an overall advantage of the RR genotype for enhanced performance in some speed and power-oriented sports. In addition, RR genotype might also favour the ability to withstand exercise-induced muscle damage, while the beneficial influence of the XX genotype on aerobic exercise performance needs to be validated in human studies. More information is required to unveil the association of *ACTN3* genotype with trainability and injury risk during acute or chronic exercise.

Keywords: Genomics; common human polymorphism; α-actinin-3 deficiency; athletic performance; muscle performance.

ABBREVIATIONS LIST

ACE: Angiotensin converting enzyme

CK: Creatine kinase

KO: knockout

mTOR: mammalian target of rapamycin

SERCA1: Sarcoplasmic/endoplasmic reticulum calcium ATPase 1

WT: Wild type

INTRODUCTION

There are two muscle sarcomeric isoforms of α-actinin; α-actinin-2, and α-actinin-3, which are encoded by *ACTN2* and *ACTN3* genes, respectively (Beggs et al. 1992). The α-actinin-2 and α-actinin-3 proteins form major components of the contractile apparatus at the Z-line and are structured similarly. However, α- actinin-2 is ubiquitously expressed in all muscle fiber types, while α-actinin-3 expression is found only in the fast type II fibers (Mills et al. 2001). This indicates that α-actinin-2 and 3 might have different physiological roles in the muscle (Lee et al. 2016). The protein α-actinin-2 plays a central role within the sarcomere for all types of locomotor activities and displacements (Ribeiro Ede et al. 2014) while the α-actinin-3 role might be more related to the generation of rapid muscle force and thus, for the production fast and explosive movements (MacArthur et al. 2007). Further the role of α-actinin-3 has been attributed to modulate the response to training, the recovery after exercise-induced muscle damage and the exercise-associated risk of injury (Pickering and Kiely 2017).

North et al. (1999) identified a common stop-codon polymorphism (rs1815739; R577X) in the *ACTN3* gene. Individuals who are homozygous for the X allele are unable to express α-actinin-3 in type II muscle fibers as opposed to those with the RX or RR genotype. Interestingly, individuals with the *ACTN3* XX genotype compensate the deficiency of α-actinin-3 with a higher expression of α-actinin-2 (Seto et al. 2011). This in turn might confer different properties to the muscle fiber altering muscle function at rest or during exercise (Eynon et al. 2011; Santiago et al. 2010). Thus, the physiological effects found in XX homozygotes are explicitly related to lack of α-actinin-3 instead of to a reduced amount of α-actinins within the muscle. Although α-actinin-2 and α-actinin-3 isoforms are almost identical in structure, subtle differences in the interaction with other proteins might have a crucial effect on both the Z-line and the entire sarcomere (Lee et al. 2016; Berman and North 2010). Around one fifth (20%) of the world population has the XX genotype (MacArthur et

al. 2007). Although α-actinin-3 deficiency is not translated into any muscle disease, the current evidence suggests that it might affect muscle physiology in athletes (Alfred et al. 2011), healthy individuals, (Broos et al. 2015) and in some clinical populations (Pickering and Kiely 2018).

Literature suggests that α-actinin-3 deficiency might play a beneficial role that would explain the perpetuation of the X allele through natural selection in human evolution. It has been postulated that the survival of the X allele has been influenced by ambient temperature (Lee et al. 2016) because its frequency in human populations increases with the distance from central latitudes (Amorim et al. 2015; Friedlander et al. 2013). This was confirmed by different researches that have found that the frequency distribution of the *ACTN3* XX genotype averages ~25% in Asians, 18% in Caucasians, 11% in Ethiopians, and 3% in US African Americans and only 1% in Kenyans (Yang et al. 2007; Scott et al. 2010; Pickering and Kiely 2017). The relatively high frequency of the XX genotype in human populations living in cold environments might be related to improved acclimatization and thermogenesis, due to increased metabolic heat generation during muscle activities (Head et al. 2015). In addition, the X allele offers enhanced metabolic efficiency (MacArthur et al. 2008; MacArthur et al. 2007) and therefore it might have persisted as a metabolically 'thrifty' allele (MacArthur and North 2004) that could have favoured hunting in environments with scarce food resources (Amorim et al. 2015).

In this review, we will discuss the recent insights into the role of α-actinin-3 in muscle, based on studies in humans and the *Actn3* knockout (KO) mouse model. We will then focus on the consequences of α-actinin-3 deficiency on human exercise performance, exercise-induced muscle damage, injury epidemiology and response to training.

CONSEQUENCES OF α-ACTININ-3 DEFICIENCY ON SPRINT/POWER-ORIENTED PERFORMANCE

The consequences of the *ACTN3* genotype on human exercise performance were first investigated in elite athletes. Initially, it was found that the *ACTN3* 577XX genotype was under represented in sprint athletes, when compared to a control population of healthy untrained individuals (Yang et al. 2003). In fact, none of the Olympic power/sprint athletes included in this first investigation by Yang et al. (2003) were α-actinin-3 deficient (XX), suggesting that this genotype was unfavourable for fast and powerful muscle contractions, at least in elite sports. This was replicated in several elite athlete cohorts where there is a higher frequency of the R allele in sprint and power disciplines (Eynon et al. 2013; Alfred et al. 2011; Kikuchi et al. 2014; Eynon et al. 2009; Roth et al. 2008; Ginszt et al. 2018; Niemi and Majamaa 2005) and demonstrated in subsequent meta-analyses ((Weyerstrass et al. 2018; Ma et al. 2013); see Table 1 for a summary). Further, recent literature suggests that the presence of α-actinin-3 might be especially advantageous for sprint, and power-based sports but not for strength-based sports disciplines (Ben-Zaken et al. 2016; Kim et al. 2014a). In addition, the role of α-actinin-3 for the generation of high-intensity muscle contractions has been confirmed in research with non-athletes. Untrained RR individuals have a higher baseline strength than their XX counterparts (Walsh et al. 2008; Clarkson et al. 2005a; Erskine et al. 2014) although this is contentious as other studies found no such differences between genotypes (Hanson et al. 2010; Norman et al. 2009). While muscle fiber composition is not affected by α-actinin-3 deficiency (Norman et al. 2014; Norman et al. 2009), the cross-sectional area of type II muscle fibers might be larger in RR than in XX individuals (Broos et al. 2016). Nonetheless, α-actinin-3 deficiency does not totally preclude the possibility of achieving high performance in power-like sports, because other cohorts of

elite power/sprint athletes have reported a normal frequency of XX individuals (Ruiz et al. 2011; Sessa et al. 2011; Wang et al. 2013; Ruiz et al. 2013).

It has been proposed that the higher capacity of RR individuals to perform in speed/power sports might be also coupled with a better response to strength, and power oriented training (Pickering and Kiely 2017). For example, Delmonico et al. (2007) found greater increases in knee extensor peak power after 10 weeks of unilateral strength training in RR men and women compared to their XX counterparts. Norman et al. (2014) reported a higher exercise-induced increase in the phosphorylation of the mammalian target of rapamycin (mTOR) and p70S6k proteins in R allele carriers compared to the XX allele carriers' in human muscle fibers, suggesting an ameliorated muscle protein synthesis in α-actinin-3 deficient individuals. The *Actn3* KO mouse, firstly generated by MacArthur et al. (2007), mimics the α-actinin-3 deficient phenotype in humans. The *Actn3* knockout mice have identical morphological characteristics to wild type (WT) mice and they compensate the lack of α-actinin-3 with α-actinin-2 (Lee et al. 2016). In this mouse model, it has been found there is a higher calcineurin activity in *Actn3* KO mice (Seto et al. 2013) that would suggest a unfavourable physiological adaptability of α-actinin-3-deficient individuals to strength and power training stimuli ((Seto et al. 2013; Garton et al. 2014; Chin et al. 1998); Table 2). Indeed, calcineurin is a cytoplasmic calcium-regulated phosphatase implicated in fiber type transformations (Dunn et al. 1999), where activation in skeletal muscle selectively up-regulates promoters of specific slow-fiber genes (Chin et al. 1998). In this context, the muscle tissue of α-actinin-3-deficient individuals theoretically might be more prone to adapt to endurance training stimuli rather than to strength or power oriented programs (Seto et al. 2013; Garton et al. 2014).

While these investigations provide mechanistic support for an increased training response to strength/power-oriented programs in individuals who are able to express α-actinin-3 (*i.e.*, those with RR or RX genotypes) this might also be modulated by other genes.

A report on the combined association of *ACTN3* and angiotensin converting enzyme (*ACE*) insertion (I)/deletion (D) genotypes in older women found that non α-actinin-3 deficient genotype (*ACTN3* RR) combined with the *ACE* DD genotype might favour training gains in muscle strength, power and functionality (*i.e.*, during the sit-stand test) after a 12-week speed/power training protocol (Pereira et al. 2013b; Pereira et al. 2013a). The effectiveness of personalized resistance training based on genetics requires further investigation as it has only proven effective in one investigation, which included a cumulative genotype score based on *ACTN3* R77X and 14 other genetic polymorphisms (Jones et al. 2016). Finally, other investigations have not found higher strength gains in RR individuals when compared to XX counterparts (Erskine et al. 2014; Clarkson et al. 2005a). Thus, the evidence available does not suffice yet to support that RR individuals are more prone to obtain benefits from strength or power-based exercise programs than XX individuals.

CONSEQUENCES OF α-ACTININ-3 DEFICIENCY ON ENDURANCE-ORIENTED PERFORMANCE

Recent literature has shown that the absence of α-actinin-3 within the skeletal muscle might be advantageous in certain and specific situations, explaining the survival of the *ACTN3* 577X homozygosity through natural selection. In mouse model studies it has been found that there is a shift towards a more efficient aerobic muscle metabolism coupled with an improved recovery from fatigue in *Actn3* KO mice *vs* WT littermates (MacArthur et al. 2008; MacArthur et al. 2007; Chan et al. 2008). *Actn3* KO mice present with a higher activity of key oxidative enzymes, especially in fast muscle fibers, such as NADH-tetrazolium reductase and succinate dehydrogenase (MacArthur et al. 2007). This indicates an enhanced capacity for fat/carbohydrate oxidation during repeated and moderate-intensity muscle contractions (Table 2). However, in humans, the potential advantage of the X allele

or the XX genotype for endurance sports performance is less clear, with some investigations reporting no increased frequency of the XX genotype in endurance athletes (Grealy et al. 2013; Guilherme et al. 2018; Papadimitriou et al. 2018; Ma et al. 2013; Lucia et al. 2006; Saunders et al. 2007; Yang et al. 2007) or even an under-representation of this genotype (Ahmetov et al. 2010; Kikuchi et al. 2016; Li et al. 2017) respect to control/untrained populations. Thus, it can be assumed that perhaps lack of α-actinin-3 does not offer a major advantage for endurance performance, at least in athletic populations (Table 1).

Regarding training adaptations, XX might be more of a 'responder' (*i.e.*, able to improve fitness outcomes) to endurance training than those with the RR genotype (Jones et al. 2016; Pickering and Kiely 2017). *Actn3* KO mice showed an improved adaptive response to 4 weeks of endurance training compared to WT mice (Seto et al. 2013). Interestingly, the muscle of WT mice did not display a shift in fiber type in response to endurance training whereas, *Actn3* KO mice had a decrease in the cross-sectional area of 2B fibers (which is the fastest muscle fiber type in mice) and an increase in 2X fibers, suggesting a "slowing" of the metabolic and physiological properties of fast fibers in this α-actinin-3 deficient mice (Seto et al. 2013). Further physiological changes within the muscle fiber produced by the lack of α-actinin-3 is higher calcineurin activity, which has been found in both *Actn3* KO mice and humans with the XX genotype (Seto et al. 2013). In this context, the muscle tissue of α-actinin-3-deficient individuals theoretically might be more prone to adapt to endurance training stimuli rather than to strength or power oriented programs (Seto et al. 2013; Garton et al. 2014). Although Silva et al. (2015) found that RR individuals presented a higher adaptive response to endurance training than those with the XX genotype (Silva et al. 2015), Magi et al. (2016) found no influence of *ACTN3* genotype on the peak oxygen consumption responses to 5 years of endurance training in young cross-country skiers. The contradictory results might be due, at least partly, to differences between studies in the training protocols implemented or in the baseline fitness levels of the participants. Future investigations in the

field should be carried out in both detrained and highly endurance trained individuals, with proper standardizations in the protocols used for training and controlling for factors that could affect trainability, such as the use of concurrent training sessions or of nutritional supplements and ergogenic aids.

THE ROLE OF α-ACTININ-3 TO REDUCE EXERCISE-INDUCED MUSCLE DAMAGE

Exercise-induced muscle damage is a physiological process that typically occurs after unaccustomed exercise, particularly if the exercise involves a large amount of eccentric contractions (Clarkson and Hubal 2002). This phenomenon is associated with muscle soreness and is thought to start with mechanical disruption of the affected fibers (e.g., as manifested by Z-line streaming), which in turn leads to an inflammatory response. Surrogate markers such as increased levels of pro-inflammatory cytokines, and leakage of intra-muscle proteins in the blood, such as creatine kinase (CK), are used to assess exercise-induced muscle damage (Yamin et al. 2010). The protective role of α-actinin-3 against damage in type II muscle fibers has been shown in several research protocols that included acute eccentric or concentric muscle actions. Individuals with the XX genotype presented with higher serum CK levels and muscle pain values than RR individuals after a protocol of eccentric knee extensions (Vincent et al. 2010). Similarly, XX soccer players showed higher serum CK concentrations than their R allele counterparts after a session of plyometric exercise (Pimenta et al. 2012). The athletes carrying the X allele presented higher reductions in jump height, and higher values of serum CK and self-reported muscle pain than RR athletes after a marathon (Del Coso et al. 2017a) or a half-ironman triathlon (Del Coso et al. 2016). XX individuals also presented with higher serum concentrations of serum myoglobin and CK than R allele carriers after an ultra-endurance adventure race (Belli et al. 2017). This

role of α-actinin-3 for defending against damaging muscle activities during endurance/ultra-endurance exercise is somewhat unexpected when considering that slow-twitch fibers are preferentially recruited during these exercise tasks (Asp et al. 1999; North 2008). Perhaps, α-actinin-3 plays a role during the eccentric phase of endurance exercise activities that confers a higher capacity to the muscle, as a whole, to resist muscle damage despite the restricted expression of this protein to fast-twitch fibers.

Other investigations did not find an association between *ACTN3* R577X genotypes and muscle damage induced by elbow flexion eccentric exercises (Clarkson et al. 2005b). Interestingly, the "repeated-bout effect", that is, performing one bout of eccentric exercise to induce an adaptation such that the muscle is less vulnerable to muscle damage in a subsequent bout of the same type of exercise (Starbuck and Eston 2012) might be more marked in XX than in RR individuals suggesting that XX might be able to undertake more frequent training sessions (Venckunas et al. 2012). This finding was reported using drop jumps as the only exercise model to induce muscle damage and therefore requires further corroboration with other forms of exercise. A recent study found that, rather than the *ACTN3* R577X variant only, it was the cumulative influence of several genetic polymorphisms (including *ACTN3* R577X) that was associated with the magnitude of muscle damage after a marathon (Del Coso et al. 2017b). This latter investigation agrees with a previous analysis in which the *ACTN3* R577X genotype, in combination with other genes, was related to an increased likelihood of suffering a clinical case of exertional rhabdomyolysis (Deuster et al. 2013). Thus, the presence of α-actinin-3 could offer a structural benefit within the skeletal muscle fiber that might reduce the damage produced in the sarcomere during exercise but with other hereditable factors involved in the phenotype of resistance against muscle fiber damage. In this regard, the upregulated production of α-actinin-2 in muscle fibers found in XX individuals (Seto et al. 2011) does not suffice to prevent a higher likelihood of muscle damage and its accompanying symptoms in α-actinin-3 deficient individuals.

α-ACTININ-3 DEFICIENCY AND MUSCLE INJURY

The influence of the *ACTN3* R577X genotype on the levels of exercise-induced muscle damage, with the latter being a physiological phenomenon, might also suggest that α-actinin-3 deficiency can be associated with a higher incidence or severity of exercise-related muscle injuries (*i.e.*, a pathological phenomenon that might sometimes be linked to initial muscle damage, muscle contractures and strains, etc). It has been reported that XX soccer players (first league division, Italy) had almost a three- and two-fold higher probability of suffering muscle injuries in general and severe muscle injuries, respectively, than their RR counterparts (Massidda et al. 2017). Yet, another study found that R allele carriers had an increased likelihood of suffering non-contact muscle injury during different sports-activities when compared to X allele carriers (Iwao-Koizumi et al. 2015). Interestingly, the authors hypothesised that the presence of α-actinin-3 would be associated with a higher magnitude of muscle strength in R allele carriers that would have produced a higher muscle strain during exercise, thereby explaining their results. In this regard, R allele carriers had increased passive hamstring stiffness compared to non-carriers, although this mechanical property did not increase the risk of strain injury (Miyamoto et al. 2018). In athletes of combat sports (judo, taekwondo, boxing), subjects with the XX genotype had a similar kinematic efficacy in the lower limbs than R allele carriers, as assessed with knee, angle and pelvis moments during a sprint with a change of direction and a vertical jump (Jung et al. 2016). Thus, the evidences that relate deficiency of α-actinin-3 with a higher risk for muscle injury during exercise are still inconsistent (Table 2).

α-ACTININ-3 DEFICIENCY AND OTHER TYPES OF INJURIES

Although the absence of α-actinin-3 in skeletal muscle fibers has not been linked with significant changes in connective or ligament tissues, dancers with the XX genotype show a higher incidence of ankle injuries than R allele carriers (Kim et al. 2014b). The XX genotype has also been associated with a higher incidence of non-contact acute ankle sprains (Qi et al. 2016; Shang et al. 2015), although this association has not been replicated by others (Kim et al. 2017a). If accurate, the higher likelihood of injury in α-actinin-3 deficient individuals might be related to a dysfunction in the capacity of the muscle to hold the joint during the action, rather than to a dysfunction of the ligament tissue (Pickering and Kiely 2017). Another factor that is intrinsically related to the risk of joint injuries during exercise is the range of motion in active joints and muscle flexibility. In this context, Zempo et al. (2016) found that RR individuals had lower trunk flexibility than X allele carriers, as measured by the sit-and-reach test. This was replicated by Kikuchi et al. (2017) in a similar investigation with untrained individuals. However, a lower trunk flexibility has been found in XX ballerinas when compared to R allele carriers (Kim et al. 2014b). In regards to other joints, a reduced range of motion in the elbow has been found in RR homozygotes (Kikuchi et al. 2018). The discrepancies in the outcomes of these investigations indicates that a clear effect of the *ACTN3* R577X genotype on muscle flexibility and range of motion is yet to be elucidated.

In addition to its presence in fast glycolytic skeletal muscle fibers, α-actinin-3 is expressed in osteoblasts. *Actn3* KO mice presented lower bone mineral density and bone formation rates per unit of bone surface when compared to WT littermates, suggesting that the lack of α-actinin-3 is associated with disruptions in mineralisation and resorption (Yang et al. 2011) . α-actinin-3 deficient humans also presented with lower levels of bone mineral density (Yang et al. 2011; Min et al. 2016), as well as higher values of serum bone remodelling markers than R allele carriers at rest (Levinger et al. 2017). This information

suggests that XX genotype may contribute to a higher likelihood of bone injury during exercise but to the date, there is no evidence for an effect of the *ACTN3* genotype on the risk or severity of bone injuries during exercise activities.

There is need for more research to clearly associate α-actinin-3 deficiency with injury risk in athletic and non-athletic populations. The use of genome-wide association studies might be effective in helping to fill the gaps of why some individuals are more susceptible to injury than others. This has already been found useful to explain higher risk of rotator cuff injury (Roos et al. 2017), Achilles tendinopathy and anterior cruciate ligament rupture (Kim et al. 2017b) and ankle injuries (Kim et al. 2017a).

SUMMARY AND CONCLUSIONS

ACTN3 R577X genotype influences human performance in several manners related to the structural, metabolic and signalling effects by the absence or presence of α-actinin-3 within the skeletal muscle. Table 3 includes an evidence-based analysis of the association between *ACTN3* genotype and different muscle phenotypes in human and animal experiments. The RR genotype, which results in the full expression α-actinin-3 in fast-twitch fibers, favours the ability to generate powerful and forceful muscle contractions, leading to an overall advantage of this genotype for performance in some speed and power sports. Although more research is needed, this genotype might also favour the ability to withstand exercise-induced muscle damage. On the other hand, theoretically a beneficial influence of the XX genotype on aerobic exercise performance remains to be corroborated in human studies. More information is required to unveil the association of *ACTN3* genotype with injury risk during acute or chronic exercise.

Most of the information included in this review refers to research on *ACTN3* genotype frequency and their association with phenotypes related to elite athletic status.

Hence, it is still too early to determine whether the information that is currently available on the *ACTN3* genotype-phenotype associations can be directly used for genetic testing in the general/amateur population. Studies on the impact of α-actinin-3 deficiency in amateur athlete populations with more ecological research protocols should be the focus of future lines of investigation.

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CONFLICT OF INTEREST

The authors of this investigation declare that there is no conflict of interest.

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Figure 1. Localisation of α-actinin in skeletal muscle.

The sarcomeric α-actinins are essential for the contractile apparatus at the Z-line because they bind and cross-link the ends of F-actin filaments from adjacent sarcomeres. While the expression of α- actinin-2 is ubiquitous in all types of muscle fibers, α-actinin-3 is restricted to fast type II fibres, suggesting a different physiological role of each isoform for muscle contraction. α-actinins are antiparallel homodimers of more than 200 kDa, comprising an actin-binding domain (ABD), a central domain of four spectrin-like repeats (SR1-4), and a C-terminal calmodulin-like domain with two pairs of EF hand motifs (EF). Adapted from (Ribeiro Ede et al. 2014).

Figure 2. Most common phenotypes related to α-actinin-3 deficiency due to homozygosity for the X allele in the ACTN3 R577X polymorphism.

Figure 1

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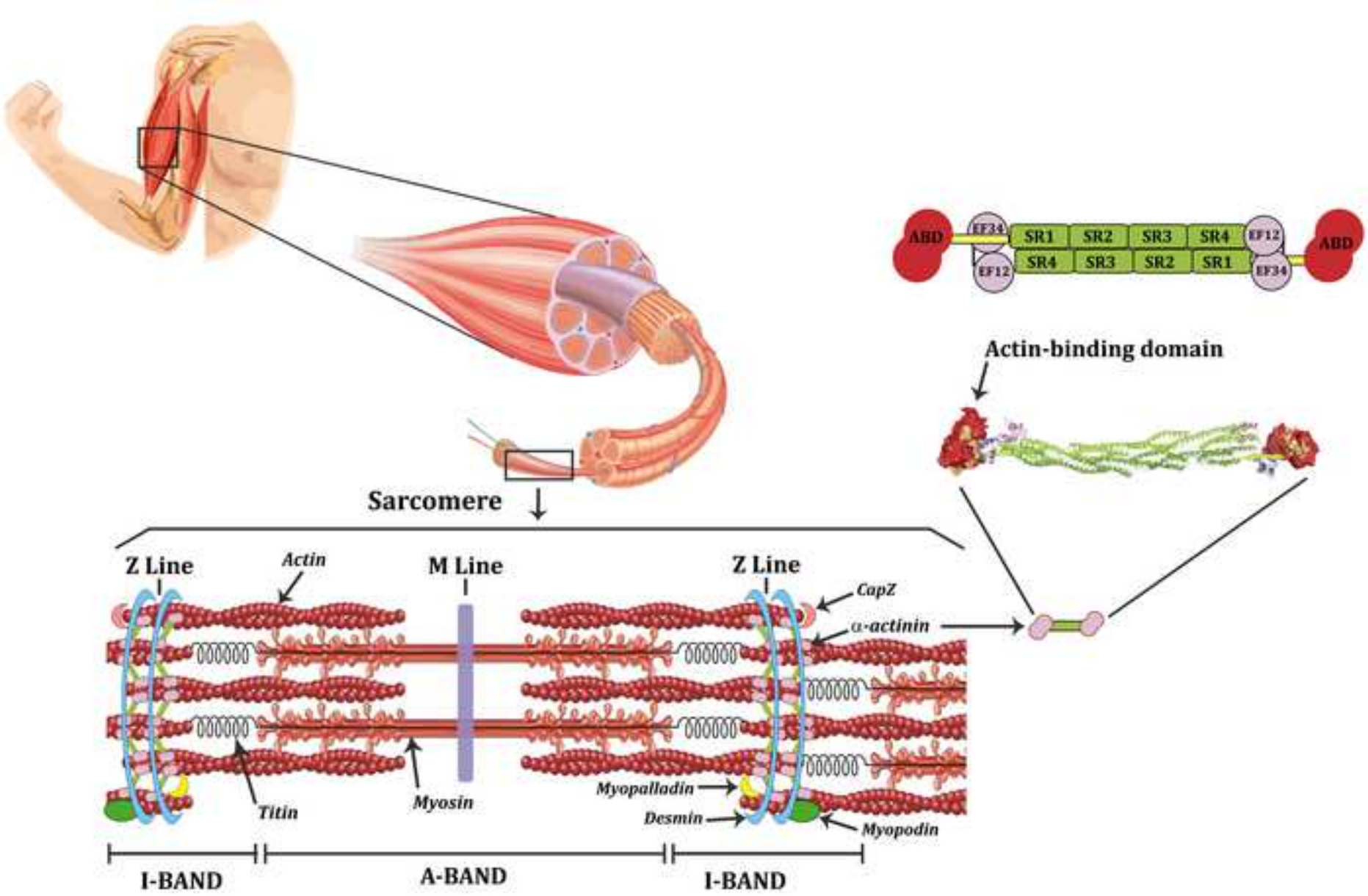

Figure 2

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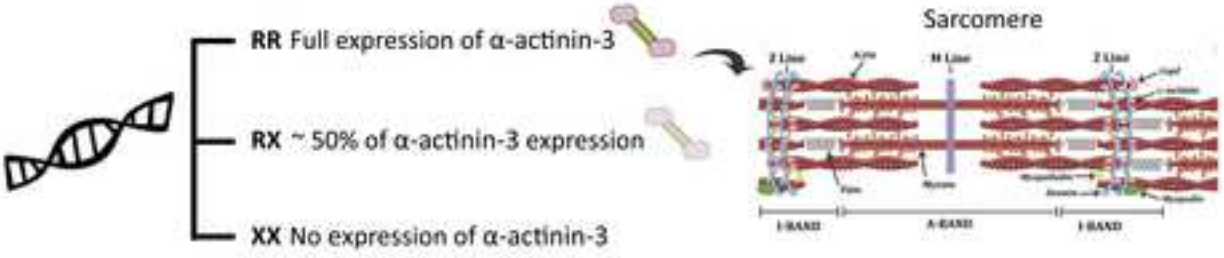
More than a "speed gene": *ACTN3* R577X genotype, trainability, muscle damage and the risk for injuries

α-actinin-3 is a bundling protein that binds and cross-links the ends of F-actin filaments to the sarcomere.

α-actinin-3 is only expressed in type II skeletal muscle fibers.

α-actinin-3 is encoded by *ACTN3* gene. A common stop-codon polymorphism (R577X) in this gene was discovered in humans.

Homozygosity for the X allele (577XX) results in the absence of α-actinin-3.



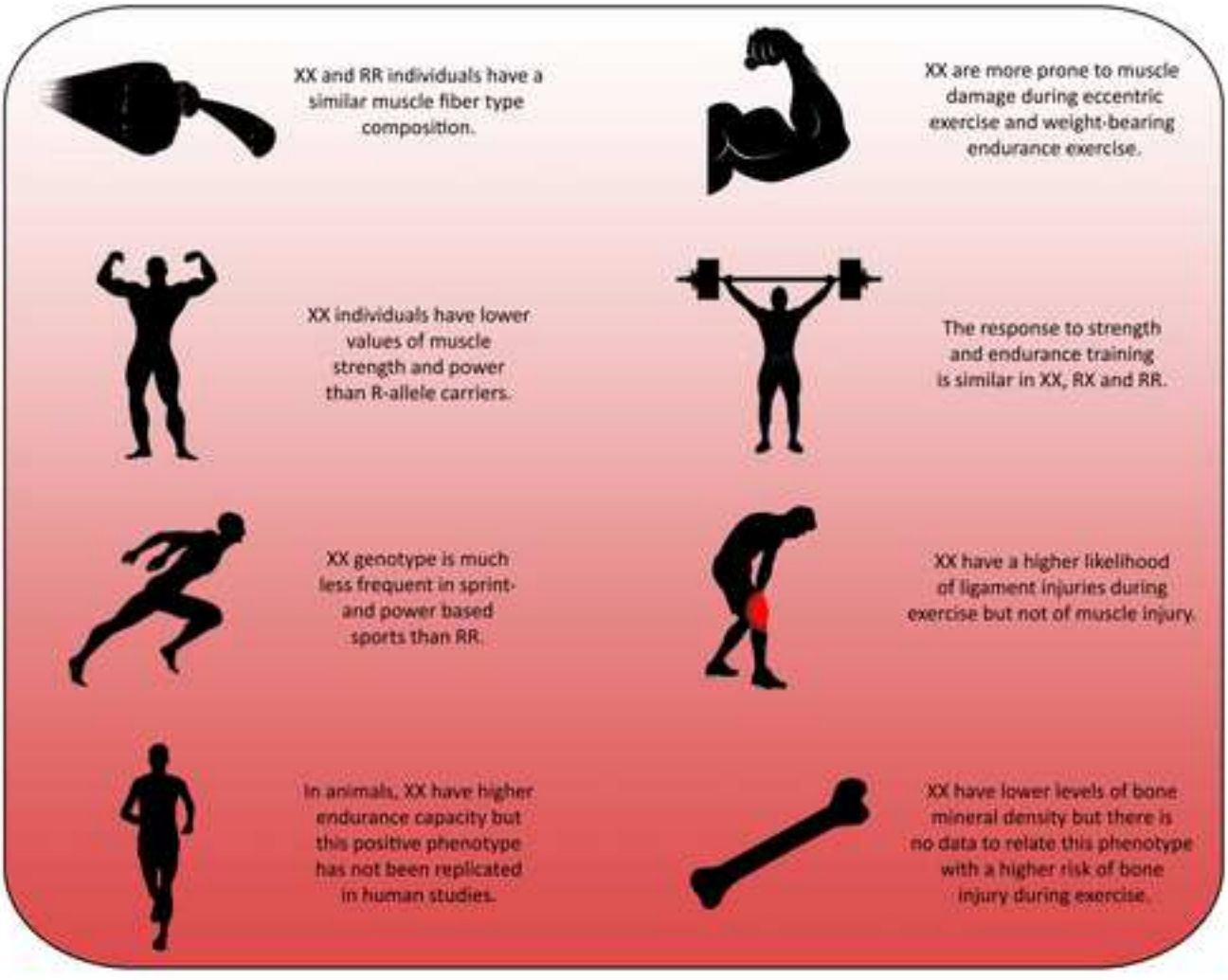


Table 1

Table 1. Summary of the physiological/performance consequences of α-actinin-3 deficiency in humans.

	Sprint/power	**Endurance**	**Muscle damage**	**Trainability**	**Risk of injury**
Overview	↓ sprint/power performance ↓ muscle strength	Mixed results for endurance performance and fatigue resistance	↑ muscle damage after eccentric exercise or weight-bearing endurance exercise	Mixed results for strength and endurance trainability	↑ risk of ligament injury and mixed results for risk of muscle injury or muscle flexibility
Proposed mechanism	↓ capacity to generate muscle force and power specially in muscle contractions that requires the recruitment of type 2 muscle fibers	↑ metabolic efficiency ↑ aerobic metabolism	↓ capacity to resist muscle strain during acute eccentric or repeated concentric contractions	↓ exercise-induced increase mTOR and p70S6k ↑ calcineurin activity	↓ capacity to resist muscle strain that could lead to reduced joint stability ↑ ligament tension
Consequence for exercise and sport	↓ frequency of XX individuals although this effect is minor in strength-based disciplines	↑ frequency of XX individuals initially found but this has been disputed in more recent investigations.	↑ levels of serum markers of muscle damage ↑ muscle pain after damaging exercise	No measurable consequences have been identified in sport	↑ frequency of XX individuals among patients with ankle joint injuries compared to their injury-free peers.

Abbreviation: mTOR, mammalian target of rapamycin

Table 2

Table 2. Summary of the physiological consequences of α-actinin-3 deficiency identified with the knock-out mouse model for the α-actinin-3 gene (*Actn3*).

	Metabolic	**Signalling**	**Structural**	**Calcium-handling**
Overview	Shift towards more aerobic metabolism	↑ adaptive response to endurance stimuli	Altered contractile properties	Altered calcium kinetics
Mechanism	↑ oxidative enzymes and ↓ glycolytic enzymes	↑ calcineurin activity	↑ expression of Z-line proteins	↑ calcium release and absorption
Physiological impact	↑endurance capacity and resistance to fatigue	↑ endurance trainability	↓ production of force	↑ production of metabolic heat
Further considerations	It is the result of changes in various enzymes	It is the result of the preferential binding of calsarcin 2 to α-actinin-2	It is the result of the preferential binding of Z-line proteins to α-actinin-2	It is the result of higher levels of SERCA1

Abbreviations: SERCA1, Sarcoplasmic/endoplasmic reticulum calcium ATPase 1.

Table 3

Table 3. Level of evidence from human and mouse model research for the effects of the α-actinin-3 deficiency on different muscle phenotype traits.

Muscle trait	Human	Mouse	Applications for exercise	References for human studies	References for mouse studies
↓ sprint/power-based performance	I	I	Genotyping can be used to determine the likelihood of success in a sprint/power-based sport	(Alfred et al. 2011; Yang et al. 2003; Weyerstrass et al. 2018; Ma et al. 2013; Eynon et al. 2013; Kikuchi et al. 2014)	(MacArthur et al. 2008; Quinlan et al. 2010; Lee et al. 2016; North 2008)
↑ training response to endurance training	V	II	Too early to use personalise endurance training based on *ACTN3* genotyping	(Silva et al. 2015; Magi et al. 2016)	(Seto et al. 2013; Chan et al. 2008)
↑ endurance performance	III	II	Genotyping does not seem useful for detection of endurance talents	(Yang et al. 2003; Silva et al. 2015; Pasqua et al. 2016; Eynon et al. 2012)	(MacArthur et al. 2007; Chan et al. 2008; Seto et al. 2013; North 2008)
↓ training response to strength training	IV	II	Too early to personalise strength training based on *ACTN3* genotyping	(Norman et al. 2014; Delmonico et al. 2007)	(Garton et al. 2014; Seto et al. 2013; Lee et al. 2016)
↑ risk of muscle damage	II	IV	Specific training might be used to ameliorate muscle damage in XX individuals	(Vincent et al. 2010; Del Coso et al. 2017a; Del Coso et al. 2016)	(Seto et al. 2011)
↑ risk of muscle/ligament injury	IV	-	Prevention plans to reduce ligament injuries in XX individuals	(Massidda et al. 2017; Kim et al. 2014b; Qi et al. 2016; Shang et al. 2015)	-
↑ risk of bone injury	IV	IV	Too early to develop prevention plants to avoid bone injuries in XX individuals	(Yang et al. 2011; Min et al. 2016; Levinger et al. 2017)	(Yang et al. 2011)

Level I: Systematic and narrative reviews, meta-analyses and high-quality prospective cohort studies with adequate power and with consistent results regarding the association of *ACTN3* genotype and the specific muscle trait. Level II: Lesser high-quality prospective cohort studies, retrospective/comparative studies or systematic reviews and meta-analysis with inconsistent results regarding the *ACTN3* genotype and the specific muscle trait. Level III: Case-control and retrospective studies with inconsistent results regarding the association of *ACTN3* genotype and the specific muscle trait. Level IV: Absence of association between the *ACTN3* genotype and the specific muscle trait or inconsistent results derived from cases series or poorly referenced investigations with no sensitivity analyses of phenotypes. Level V: Expert opinion. Adapted from (Burns et al. 2011). Abbreviation: *ACTN3*, α-actinin-3 gene.

Author Contribution Statement

JDC, PH, NE and AL conceived and designed the organization of the review. JDC wrote the manuscript. DH, PH, NE and AL revised the manuscript critically and added intellectual content. LMP designed the illustrations. All authors read and approved the final version of the manuscript.